Differences in proleptic and epicormic shoot structures in relation to water deficit and growth rate in almond trees (Prunus dulcis)

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Differences in proleptic and epicormic shoot structures in relation to water deficit and growth rate in almond trees (*Prunus dulcis*)

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• *Background and Aims* Shoot characteristics differ depending on the meristem tissue that they originate from and environmental conditions during their development. This study focused on the effects of plant water status on axillary meristem fate and flowering patterns along proleptic and epicormic shoots, as well as on shoot growth rates on 'Nonpareil' almond trees (*Prunus dulcis*). The aims were (1) to characterize the structural differences between proleptic and epicormic shoots, (2) to determine whether water deficits modify shoot structures differently depending on shoot type, and (3) to determine whether shoot structures are related to shoot growth rates.
• *Methods* A hidden semi-Markov model of the axillary meristem fate and number of flower buds per node was built for two shoot types growing on trees exposed to three plant water status treatments. The models segmented observed shoots into successive homogeneous zones, which were compared between treatments. Shoot growth rates were calculated from shoot extension measurements made during the growing season.
• *Key Results* Proleptic shoots had seven successive homogeneous zones while epicormic shoots had five zones. Shoot structures were associated with changes in growth rate over the season. Water deficit (1) affected the occurrence and lengths of the first zones of proleptic shoots, but only the occurrence of the third zone was reduced in epicormic shoots; (2) had a minor effect on zone flowering patterns and did not modify shoot or zone composition of axillary meristem fates; and (3) reduced growth rates, although patterns over the season were similar among treatments.
• *Conclusions* Two meristem types, with different latency durations, produced shoots with different growth rates and distinct structures. Differences between shoot type structure responses to water deficit appeared to reflect their ontogenetic characteristics and/or resource availability for their development. Tree water deficit appeared to stimulate a more rapid progression through ontogenetic states.

Key words: Almond tree, branching pattern, epicormic shoots, flowering, hidden semi-Markov model, proleptic shoots, *Prunus dulcis*, shoot growth, water deficit.

INTRODUCTION

Plants with polyaxial structure/architecture have different axis types that can be distinguished by their morphological and/or reproductive characteristics (Hallé *et al.*, 1978; Barthélémy and Caraglio, 2007). In fruit trees, shoot polymorphisms (for instance differences between short and long shoots) originate from changes in endogenous processes (Costes *et al.*, 2006). Depending on the species, proleptic shoots that develop from vegetative buds after they have gone through dormancy (Hallé *et al.*, 1978) can be composed only of preformed organs that are formed within the dormant bud (Thorp *et al.*, 1994; Sabatier and Barthélémy, 2001) or by preformed and neoformed organs that are formed and extended after bud break (Remphrey and Powell, 1984; Gordon *et al.*, 2006*a*). Proleptic shoots can have distinct structural pattern characteristics depending on genotype (Costes and Guédon, 1997, 2002), size of the shoots (Fournier *et al.*, 1998) and age of the tree when shoots develop (Renton *et al.*, 2006). Epicormic shoots are neoformed shoots that are produced from latent buds (Kozlowski and Pallardy, 1997) that remain dormant until a signal causes an epicormic shoot to grow (Kerr and Harmer, 2001; Gordon *et al.*, 2006*b*). This shoot type has been described as being more vigorous, thicker, and having longer internodes and fewer flower buds than proleptic shoots (Yamashita, 1971; Gordon *et al.*, 2006*b*). If developed after pruning, epicormic shoots reproduce the basic structure of the part of the tree that was lost, and thus can reiterate the lost structure (Barthélémy and Caraglio, 2007). Although studies have been conducted on the effect of plant water status on extension growth of proleptic shoots (Romero *et al.*, 2004) and epicormic shoots (Basile *et al.*, 2003), less attention has been paid to the potential effects of water deficit on the branching and flowering patterns along these two types of shoots.

Among the numerous plant responses to environmental conditions, shoot growth is one of the plant processes that is most sensitive to soil water availability (Bradford and Hsiao, 1982). In almond (*Prunus dulcis*), low plant water status has been related to reduced tree size (Hutmacher *et al.*, 1994; Shackel *et al.*, 2000), shoot growth rates and final shoot lengths (Romero *et al.*, 2004). Goldhamer and Viveros (2000) reported that bloom density decreased with increasing water deprivation during bud differentiation in the growing season preceding

bloom. In peach, a fruit tree closely related to almond, plant water status has been associated with reduced stem biomass dry weights (Steinberg *et al.*, 1990), shoot extension growth (Berman and DeJong, 1997), number of nodes per shoot (Girona *et al.*, 2005) and sylleptic branching (elongation from axillary meristems without a period of dormancy) on proleptic shoots (Hipps *et al.*, 1995). Since water availability can affect growth and density of branching and flowering, patterns of axillary bud fate and flower bud number per node along the shoots may be also affected by water deficit.

Distinctive branching patterns are assumed to result from the control exerted by the shoot apex on the axillary meristems (Cline, 1994). Flowering has been linked to apical growth rate and branching patterns (Kervella *et al.*, 1995; Fournier *et al.*, 1998). Studies on shoot structure, in terms of branching and flowering patterns, have aimed to identify patterns resulting from internal processes, separately from the plasticity induced by external factors. In recent years, mathematical models have been developed to quantify shoot structure based on qualitative botanical descriptions and to deepen the understanding of the processes that control shoot structure (Costes and Guédon, 1996, 1997). These modelling methods are both structural and probabilistic, since data collected on axillary productions take the form of sequences along the parent shoots that are highly structured but with some heterogeneity among individuals. A specific class of Markovian models, referred to as hidden semi-Markov models (HSMMs), has been useful to identify zones of homogeneous axillary production along shoots and to detect transitions between zones (Guédon *et al.*, 2001). This methodology has been used to represent the axillary production patterns on shoots of fruit trees under homogeneous conditions (Costes and Guédon, 1996, 1997, 2002; Fournier *et al.*, 1998). However quantitative changes in shoot structure due to external factors, such as water deficit, have not been investigated.

The present study aimed to identify whether different types of shoots (proleptic and epicormic) have different branching and flowering structures, and how their structures are affected by plant water deficit and shoot growth rate. In this case, the term 'structure' of the shoot refers to the pattern of axillary meristem fates and the number of flower buds per node along the shoot. In this study, the structures of proleptic and epicormic shoots in 'Nonpareil' almond trees were evaluated on trees with different plant water status by building an HSMM for each shoot type. It was hypothesized that the structures of these shoots would show distinctive patterns since they originated from meristem tissue with different periods of latency and were located in different positions within the trees. It was also expected that water deficit would modify the structure of both shoot types and the modifications would be unique to the type of shoot. In addition, shoot structures within the shoot would be associated with shoot growth rates at the time when specific nodes were being formed.

MATERIALS AND METHODS

This work was conducted during the growing season of 2010 and in spring of 2011 in a commercial almond (*Prunus dulcis*) orchard planted in 2007 in California's Central Valley near Firebaugh (36 ° 51′ N, 120 °19′ W). 'Nonpareil', the main scion cultivar in California, grafted onto 'Nemaguard' rootstock, was evaluated in this study.

Four blocks in different parts of the orchard were selected for the study. Each of the blocks contained three sets of four trees that received three levels of irrigation to create three plant water status treatment sub-plots. The plant water status treatments were established in the sub-plots by using two different sprinkler nozzles (Fan-Jet, Bowsmith Inc., Exeter, CA, USA) and different line pressures with two sprinklers on each side of a tree in the planting row. In the high water status treatment (HWST) the trees did not experience any significant, measurable water stress. This treatment was applied using nozzles that provided 31·8 L h^{-1} at 20 p.s.i. Two water deficit treatments were established by using smaller nozzles operating at two different water pressures: the medium water status treatment (MWST) with 22·7 L h^{-1} at 20 p.s.i. and the low water status treatment (LWST) with 16·3 L h^{-1} at 10 p.s.i. All three plant water status sub-plots were irrigated on the same irrigation schedule. Differences in plant water status were monitored throughout the season by weekly measurements of midday stem water potential (McCutchen and Shackel, 1992) using a pressure chamber (Model 3005, Soil Moisture Equipment Co., Santa Barbara, CA, USA). The midday stem water potential was evaluated using a single leaf close to the trunk in each monitored tree, following the recommendations by Fulton *et al.* (2001). The baseline midday stem water potential, which corresponds to a physiological index of plants without water stress for specific air temperature and relative humidity conditions (Shackel *et al.*, 1997), was calculated at the time of midday stem water potential measurements from the air temperature and relative humidity, which were recorded with a thermo-hygrometer (Model CMM880, Mannix Co., Lynbrook, NY, USA). The means of treatment midday stem water potentials and baseline midday stem water potentials were separated using Tukey's range test ($P < 0{\cdot}05$).

In February 2010, a south-west-oriented scaffold was pruned in every tree to promote the development of epicormic shoots (Kerr and Harmer, 2001). In each tree, three proleptic shoots that grew from terminal buds in the upper part of the tree canopies and three epicormic shoots that grew in response to the pruning from the most distal latent buds of a pruned main structural branch were tagged for measurements (12 proleptic and epicormic shoots from the trees in each treatment sub-plot). Length and the number of new nodes of the shoots were registered approximately every 10 days throughout the growing season. Shoot growth rates at different dates were estimated from shoot length measurements and the number of days between evaluations. Then, the growth rates per node along shoots were estimated from the total number of nodes per date and the growth rate per date.

In February 2011, 36 proleptic shoots and 32 epicormic shoots per water status treatment that were undamaged were selected for morphological description. This description was done at the metamer level, the basic element of plant structure, which consists of an internode and a node with leaves and axillary meristems (White, 1979). Every metamer was described in the same direction as the axillary productions developed when the shoots were growing (i.e. from the base to the tip of the shoots), using two variables: the fate of the axillary meristem and the number of flower buds per node. For the axillary meristem fate variable, each node was categorized according to one of the following observations: (1) blind node (axillary meristem fails to develop

a bud); (2) vegetative bud; (3) sylleptic shoot; or (4) central flower bud (flower bud axillary to the leaf). The variable describing the number of flower buds per node included flower buds derived from the axillary meristem and the flower buds on a sylleptic shoot that developed at that node.

For data analysis, the two observed variables for each node along the shoot were coded as a bivariate discrete sequence indexed by the node rank. The axillary meristem fate variable corresponded to a qualitative variable (i.e. blind node, etc.). The number of flower buds per node, which is a quantitative variable, was coded from 0 to 3. Production of three or more flower buds per node developed on sylleptic shoots was not common; therefore, when three or more flower buds were observed at a node, they were coded as 3.

For an exploratory analysis of shoots without regard to structure, the frequency distributions of axillary meristem fates and of the numbers of flower buds per node were extracted from the observed bivariate sequences for each treatment to evaluate the composition of the shoots. Fisher's exact test for contingency table analysis ($P < 0{\cdot}05$) was used to compare axillary meristem fates across plant water status treatments, since this was a qualitative variable, and frequency was < 5 for > 20 % of the axillary meristems and treatment combinations. ANOVA by ranks with the global Kruskal–Wallis test ($P < 0{\cdot}05$) was used to compare the number of flower buds per node across water status treatments since this was a quantitative variable, but these data were not normally distributed.

To conduct a structural analysis, VPlants software (release 0·9), part of the OpenAlea platform (Pradal *et al.*, 2008), the successor of AMAPmod (Godin *et al.*, 1997), was used for (1) exploratory analysis of the bivariate sequences, (2) model building and (3) extracting patterns from the models. The sequences were oriented in the same direction as data were collected. Empirical intensity distributions were extracted from the sequences of proleptic and epicormic shoots in each plant water status treatment. These types of distribution indicated the probability of each observation for a given observed variable for each node rank. Using these distributions, different zones of homogeneous observations were identified along the shoots. These types of shoot structure have been modelled previously with HSMMs by Costes and Guédon (1997, 2002). In addition, the intensity distributions showed that the proleptic and the epicormic shoots had similar patterns across plant water status treatments. On this basis, it was assumed that the composition of the homogeneous zones, in terms of axillary meristem fate and number of flower buds per node, was not affected or was only slightly affected by water deficit and that every zone could be present along the shoots in all plant water status treatments, but the presence and length of a determined zone would depend on the plant water status. Thus, the observed bivariate sequences of each plant water status treatment were grouped together to estimate one global HSMM for each shoot type without considering plant water status treatments.

The HSMMs were two-scale models in which the coarse scale represented the succession and the number of nodes of homogeneous zones and the fine scale represented the composition of each successive zone (Guédon *et al.*, 2001). At the coarse scale, the number and succession of zones were represented by a Markov model. The sub-model combining the Markov model with the occupancy distributions representing the zone lengths (in number of nodes) was a semi-Markov model. At the fine scale, the axillary productions observed in a given zone were characterized by two observation distributions, one for the axillary meristem fate and the other for the number of flower buds per node. It was assumed that the axillary productions observed at a given node depended only on the non-observable zone at the same node. The complete model incorporating these observation distributions was an HSMM. This model was defined by four subsets of parameters that provided information about the shoot structure: (1) initial probabilities that determined the first zone at the base of the shoots; (2) transition probabilities that described the succession of zones along the shoots; (3) occupancy distributions that modelled zone length; and (4) observation distributions that represented the mixture of observations within each zone (Costes and Guédon, 1997, 2002; Renton *et al.*, 2006).

The initial HSMM relied on assumptions that were made from the intensity distributions regarding the number of homogeneous zones in the shoots, the succession of zones and the presence of observation types in each zone. The succession of zones was modelled with a unidirectional (or left–right) Markov model, i.e. only transitions from one zone to the next were allowed, but transitions from a given zone to the previous ones were forbidden. The presence of each observation type was allowed in every zone except in the first zone, where only blind nodes could be observed. Then, the global HSMMs were estimated using an iterative algorithm that maximized the likelihood of the observed sequences, starting with the hypotheses established in the initial model. Theoretical distributions computed from the estimated model parameters were plotted along with the empirical distributions to evaluate estimated models (Guédon *et al.*, 2001).

From the global HSMMs for the two shoot types, specific parameters for the shoots from each plant water status treatment were extracted according to the methodology proposed in Renton *et al.* (2006). To do this, the most probable state sequence of each observed bivariate sequence was computed using the global HSMM. This new sequence corresponded to the optimal segmentation in homogeneous zones of the observed sequence. The most probable state sequences, along with bivariate sequences of each shoot, were grouped according to the plant water status treatment. Then, segmentation of each shoot was used to obtain zone occurrence along the shoots and zone lengths by treatment. Zone composition was also analysed, but the results are provided as Supplementary Data.

Zone occurrence (binary variable, non-occurrence/occurrence of a zone) was compared among treatments using ANOVA by ranks with the global Kruskal–Wallis test ($P < 0{\cdot}05$) and the Wilcoxon–Mann–Whitney test ($P < 0{\cdot}05$) between pairs of plant water status treatments. The same approach was used for the evaluation of shoot and zone lengths, since these data were not normally distributed. All statistical tests were conducted with the Statistical Analysis Systems software (Version 9·2; SAS Institute, Cary, NC, USA).

RESULTS

Plant water status

As expected, midday stem water potential values differed among irrigation treatments through most of the season [two-way

ANOVA for each date ($P < 0{\cdot}05$); data not presented]. Midday stem water potentials were similar between the HWST (no water deficit) and the two water deficit treatments only at the beginning of the growing season (21 April 2010), when soil was at water-holding capacity because of spring rains and early irrigations (Fig. 1). HWST midday stem water potentials were equal to or above baseline except on two evaluation dates immediately before irrigation events (24 June and 19 July 2010) and just prior to harvest (20 August 2010). The MWST was similar to the HWST until 21 April 2010 and was above or equal to baseline until 3 May 2010. After 21 April 2010, MWST midday stem water potentials were lower than HWST and were below or equal to baseline. Trees in the LWST had the lowest midday stem water potentials throughout the season, but were above or equal to baseline until 1 May 2010, after which they were below baseline (Fig. 1).

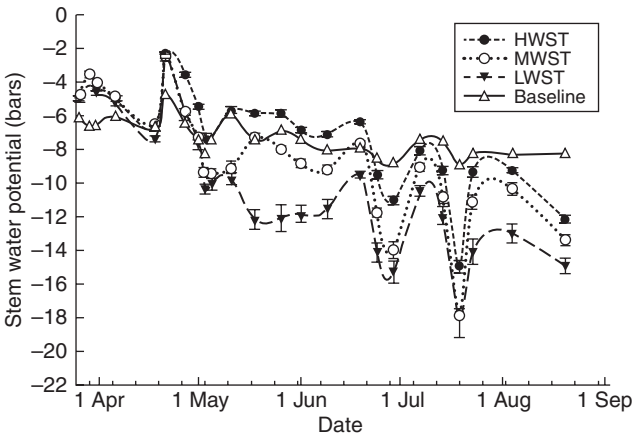

FIG. 1. Seasonal pointwise average midday stem water potential of almond trees under the high (HWST), medium (MWST) and low (LWST) plant water status treatments and the estimated baseline (non-stressed value) for the crop. Vertical bars represent s.e.

Proleptic shoots

The relative frequency of axillary meristem fates was not affected by plant water status treatment ($P = 0{\cdot}87$) (Fig. 2A). However, the total number of nodes on shoots in each of the treatments decreased with water deficit (2736, 2085 and 1776 for HWST, MSWT and LWST, respectively; $P < 0{\cdot}0001$). The reduction in the number of nodes mainly consisted of a reduction in the number of nodes that had vegetative buds, and to a lesser degree of blind nodes and nodes that had sylleptic shoots (data not shown). The number of flower buds per node was not affected by the two water deficit treatments ($P = 0{\cdot}21$) (Fig. 2B). Numerically, however, shoots in the least water-stressed trees had a 10 % lower proportion of nodes without flowers and an increased proportion of nodes with two or more flower buds (Fig. 2B).

The HSMM built for the proleptic shoots had seven states corresponding to six successive zones plus a final state that represented the terminal bud (Fig. 3A). This model was characterized by an almost deterministic succession of states (initial probability in the first state and transition probabilities between consecutive states were close to 1), the only exceptions being the probability of omitting Zones 2 and 3 in 18 % of the shoots. Each zone was defined according its composition of axillary meristem fates and number of flowers: (1) Zone 0, at the bottom of the shoots, had only blind nodes; (2) Zone 1 mainly had vegetative buds, with 55 % of them lacking flower buds; (3) Zone 2 was a mixture of sylleptic shoots and vegetative buds where 97 % of the nodes had flower buds; (4) Zone 3 was similar to Zone 1, but had more nodes with flower buds (73 %); (5) Zone 4 mainly contained vegetative buds mixed with some blind nodes; (6) Zone 5 had blind nodes and central flower buds; and (7) Zone 6 was the terminal bud.

Differences in shoot structure due to the plant water status treatments were found when comparing the zone occurrence and length of each zone (Table 1). Zone occurrence was similar among treatments except for Zone 1, which occurred significantly less often in the lowest water status treatment. The first zone, with only blind nodes, had fewer nodes on shoots in the HWST. In contrast, shoots in this treatment had more nodes in

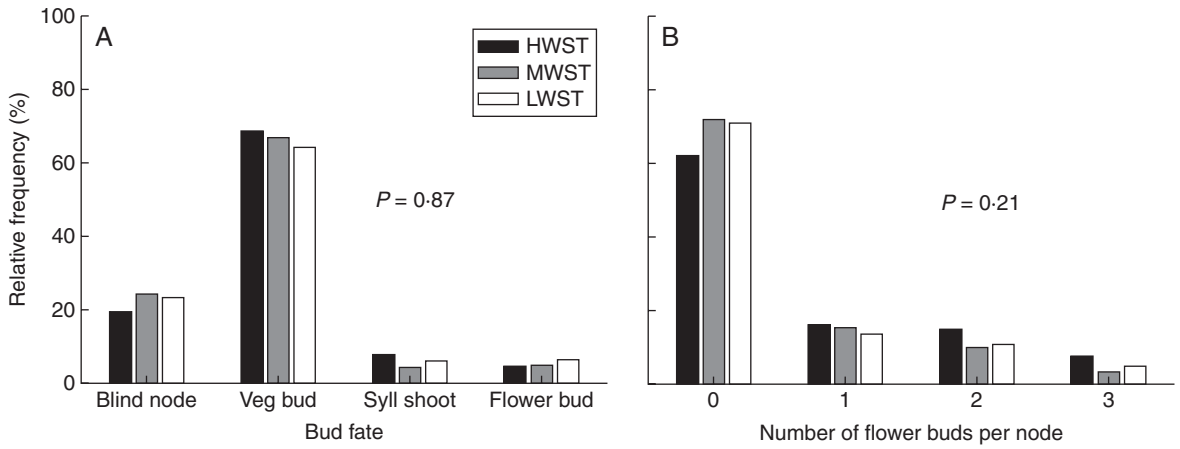

FIG. 2. Relative frequency distributions of axillary meristem fates (A) and the number of flower buds per node (B) on proleptic shoots growing in the high (HWST), medium (MWST) and low (LWST) plant water status treatments. Fisher's exact test ($P > 0{\cdot}05$) indicated no significant axillary meristem fate differences among treatments. Kruskal–Wallis ANOVA by ranks ($P > 0{\cdot}05$) indicated no significant differences in the number of flower buds per node among treatments.

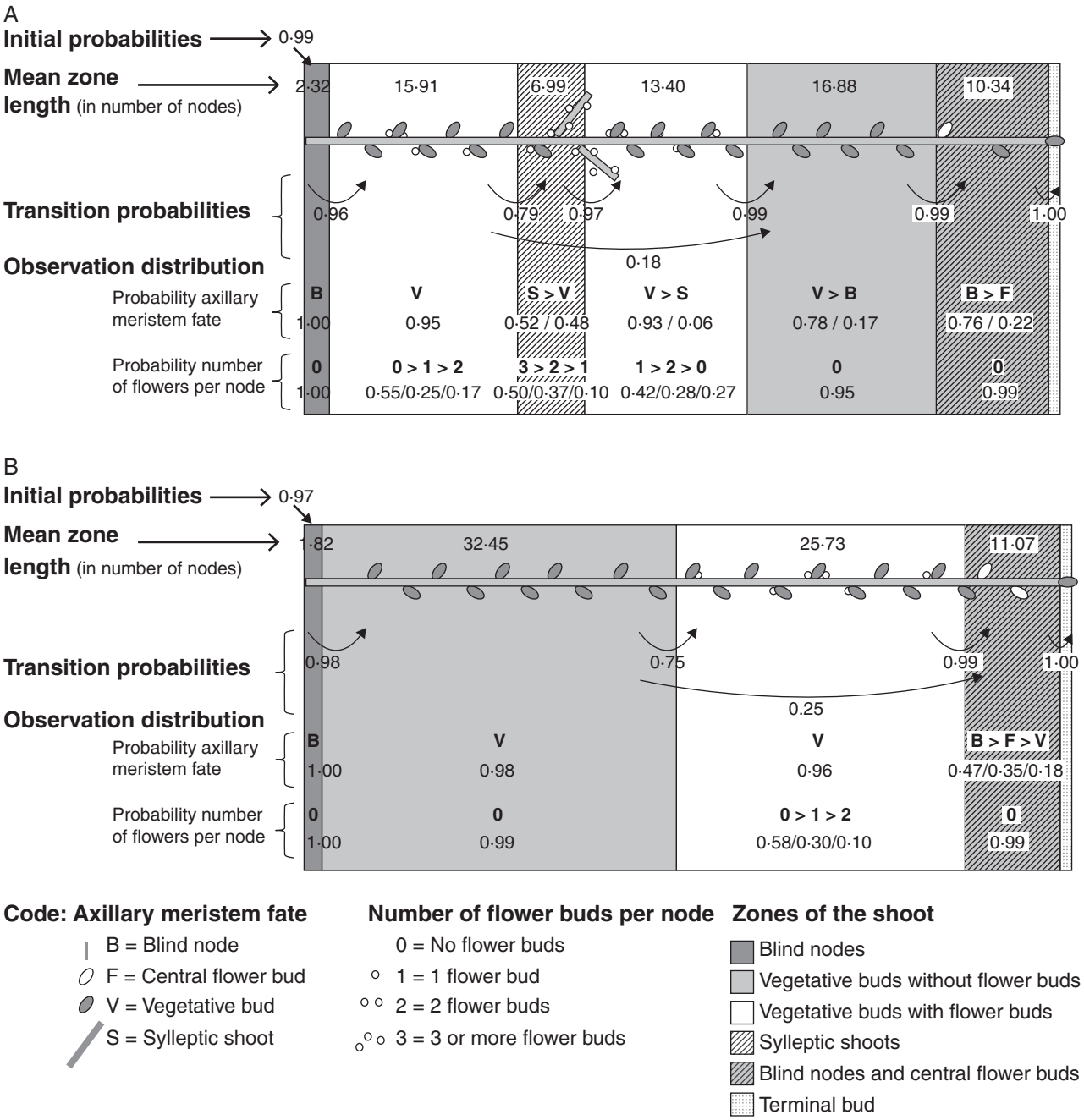

FIG. 3. Schematic representation of the global hidden semi-Markov models for the pooled sample of proleptic shoots (A) and epicormic shoots (B) for high (HWST), medium (MWST) and low (LWST) plant water status treatments. Diagrams show observations that had probabilities greater than 0·05.

the two following zones. The zones towards the end of the shoots had similar numbers of nodes across treatments.

Overall, the relative frequency of axillary meristem fates within zones was not affected by plant water status treatment. However, water deficit did change the proportion of number of nodes with flower buds in Zone 2 (Supplementary Data Tables S1 and S2).

Shoot growth rates increased markedly during the formation of the first 20 nodes (Fig. 4), and were similar among treatments during the absence of significant differences in midday stem water potentials (Fig. 1). From about node 20 to node 30, the growth rate decreased rapidly whatever the water status treatment, but trees in the HWST maintained higher growth rates than trees in the two water deficit treatments. After two or three measurement periods without much variation in shoot growth rate, a second cycle of variation in shoot growth rate was observed: shoot growth rates increased between node 30 and node 45/50, and then decreased until the end of shoot growth. Throughout this second cycle, the rate of growth was higher in the HWST than in the two water deficit treatments, but there were smaller differences between the two water deficit treatments. The sylleptic shoot zone was formed during the period of most rapid growth,

TABLE 1. *Zone occurrence and mean zone length extracted for high (HWST), medium (MWST) and low (LWST) plant water status treatments for proleptic and epicormic shoots*

		Zone occurrence (%)			Mean zone length (no. of nodes)		
		Treatment			Treatment		
Shoot	Zone	HWST	MWST	LSWT	HWST	MWST	LWST
Proleptic	0	100·0[a]	100·0[a]	97·2[a]	2·00[b]	2·44[a]	2·54[a]
	1	100·0[a]	100·0[a]	88·9[b]	21·86[a]	14·69[ab]	11·42[b]
	2	80·6[a]	69·4[a]	77·8[a]	10·38[a]	4·20[b]	4·18[b]
	3	83·3[a]	69·4[a]	75·0[a]	17·87[a]	13·20[a]	12·52[a]
	4	100·0[a]	100·0[a]	100·0[a]	17·81[a]	18·19[a]	15·58[a]
	5	100·0[a]	100·0[a]	97·2 [a]	12·25[a]	10·92[a]	8·40[a]
Epicormic	0	96·9[a]	96·9[a]	96·9[a]	1·68[a]	1·94[a]	1·90[a]
	1	100·0[a]	100·0[a]	93·8[a]	30·47[a]	35·72[a]	33·93[a]
	2	90·6[a]	71·9[ab]	62·5[b]	26·55[a]	24·61[a]	24·20[a]
	3	100·0[a]	100·0[a]	100·0[a]	9·50[a]	11·63[a]	11·16[a]

Significant differences ($P < 0·05$, Wilcoxon–Mann–Whitney test) between pairs of plant water status treatments are indicated by different letters.

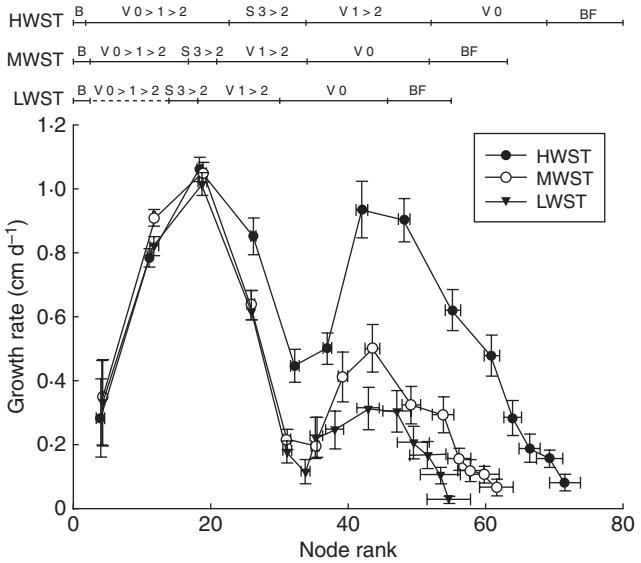

FIG. 4. Growth rate of proleptic shoots and shoot zones according to the hidden semi-Markov model for the high (HWST), medium (MWST) and low (LWST) plant water status treatments. Numbers indicate number of flower buds per node, from 0 (no flower bud) to 3 (three or more flower buds). Lines above the plot represent mean zone lengths and the dashed line for LWST indicates significantly lower occurrence probability in Zone 1. Letters indicate main observations within a zone (B, blind node; V, vegetative bud; S, sylleptic shoot; F, central flower bud).

with growth rates >0·8 cm day^{-1}, but also corresponded to the period of decrease in growth rate after the highest growth rate was reached, as observed in the HWST. The nodes with vegetative buds (V; Zones 1, 3 and 4) were formed during periods of intermediate growth rate (between 0·3 and 0·8 cm day^{-1}). The nodes of the zones that had blind nodes or central flower buds (B or F; Zones 0 and 5) were associated with lowest shoot growth rates (<0·3 cm day^{-1}). The nodes with vegetative buds associated with higher probabilities of flower buds (V 1 > 2; Zone 3) were formed when the growth rates were decreasing and were lower than when the nodes with vegetative buds without flower buds (V 0; Zone 4) were formed.

Epicormic shoots

As with proleptic shoots, axillary meristem fate was not affected by the plant water status treatments ($P = 0·96$) (Fig. 5A). Contrary to the case for proleptic shoots, the total number of nodes on shoots in each of the treatments was not significantly affected by water status treatment (2101, 2138 and 1918 for HWST, MSWT and LWST, respectively; $P = 0·36$). The number of flower buds per node was also not significantly affected by the two water deficit treatments ($P = 0·06$) (Fig. 5B). Numerically, however, shoots in the least water-stressed trees had a 10 % lower proportion of nodes without flowers and an increased proportion of nodes with two or more flower buds (Fig. 5B).

The HSMM estimated for epicormic shoots had five states and was characterized by an almost deterministic succession of states, the only exception being the omission of the third zone in 25 % of the shoots (Fig. 3B). The zones were defined as follows: (1) Zone 0 had only blind nodes; (2) Zone 1 included mainly vegetative buds with no flower buds; (3) Zone 2 had mainly vegetative buds and few sylleptic shoots (3 %), and 42 % of the nodes had flower buds; (4) Zone 3 was a mixture of blind nodes, central flower buds and vegetative buds; and (5) Zone 4 indicated the shoot terminal bud.

Only the occurrence of Zone 2 was significantly lower in the LWST in comparison with the non-water-deficit treatment (HWST) (Table 1). The length of the zones was not affected by the two water deficit treatments (Table 1).

The effect of water deficit on the relative frequencies of axillary meristem fates and the number of flower buds per node in the different zones along the epicormic shoots was similar to the effect described for proleptic shoots (Supplementary Data Tables S1 and S2).

As with proleptic shoots, shoot growth rates exhibited two successive cycles/phases separated by a period of low values at node 45 for HWST and node 40 for the two water deficit treatments (Fig. 6). However, the growth rates of epicormic shoots were higher than the growth rates of proleptic shoots (Fig. 4). The highest growth rates in the first cycle were reached around node 30 in all treatments and the rate of HWST was higher than the two water deficit treatments. Differences in shoot growth rates

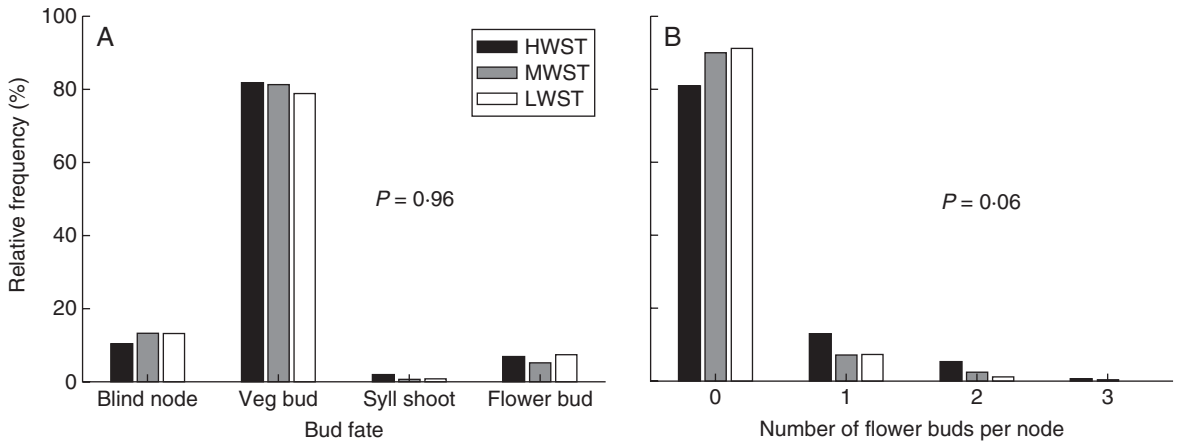

FIG. 5. Relative frequency distributions of the axillary meristem fates (A) and number of flower buds per node (B) on epicormic shoots growing in the high (HWST), medium (MWST) and low (LWST) plant water status treatments. Fisher's exact test ($P > 0{\cdot}05$) indicated no significant axillary meristem fate differences among treatments. Kruskal–Wallis ANOVA by ranks ($P > 0{\cdot}05$) indicated no significant differences in the number of flower buds per node among treatments.

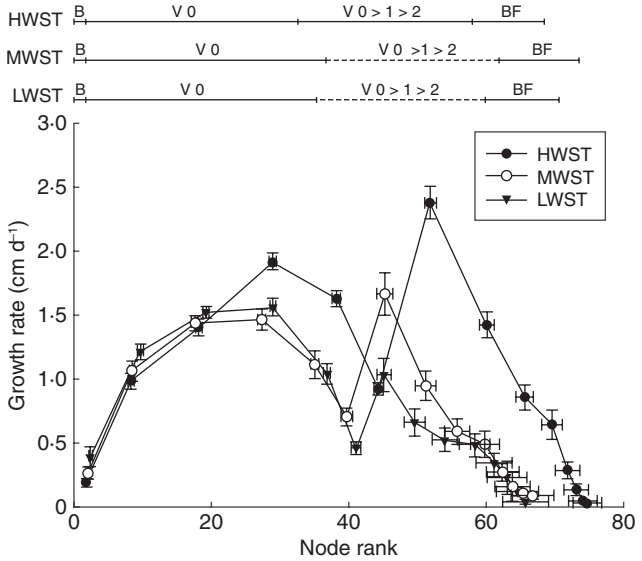

FIG. 6. Growth rate of epicormic shoots and shoot zones according to the hidden semi-Markov model for the high (HWST), medium (MWST) and low (LWST) plant water status treatments. Numbers indicate number of flower buds per node, from 0 (no flower bud) to 3 (three or more flower buds). Lines above the plot represent the mean zone lengths and dashed lines indicate zones with significantly lower zone occurrence probabilities. Letters indicate the main observation with a zone (B, blind node; V, vegetative bud; F, central flower bud).

among treatments were observed after node 20 to the end of shoot growth, but the differences between the two water deficit treatments were relatively small. Nodes with vegetative buds were formed throughout the season, shoot growth rate being $>0{\cdot}5$ cm day^{-1}. Also, few sylleptic shoots (3 %) and flower buds were observed in Zone 2 in the second cycle, with high growth rates (from 1 to 2·5 cm day^{-1}) after the slowing growth period. Low growth rates ($<0{\cdot}5$ cm day^{-1}) were associated with periods when blind nodes and nodes with central flower buds were formed. Low growth rates were also associated with the period corresponding to the formation of the central part of the shoot; this is potentially a period of transition between two zones.

DISCUSSION

Initial analysis of shoot structure using intensity probability distributions indicated differences between proleptic and epicormic shoots and some modifications due to water deficit. From this analysis, it was possible to identify a succession of zones along the shoots that differed in the fate of axillary meristems and in the number of flower buds per node. This type of structure was further explored and modelled using a global HSMM for each shoot type across the three water status treatments. By describing distinctive zones of the shoots, these two-scale models provided a level of representation intermediate between nodes and shoots, and a method to compare shoot structures (Fournier *et al.*, 1998).

Both shoot types exhibited highly structured development, with a succession of zones almost deterministic and each zone being characterized by a specific observation distribution. Even though the epicormic and proleptic shoots had similar numbers of nodes, the structure of epicormic shoots was simpler (i.e. they had fewer zones). Proleptic shoots developed more zones in the middle part of the shoot than epicormic shoots did. However some similarities were also found between the two shoot types, with blind nodes observed at their base, and a mixture of central flower buds and blind nodes observed before the terminal bud. Also, Zones 1 and 2 had some correspondence between these two types of shoot. Zone 1 had mainly vegetative buds, but with more flower buds on proleptic shoots. Zone 2 was a mixture of sylleptic shoots and vegetative buds, but the probability of sylleptic shoots was lower in epicormic shoots (only 0·03). Proleptic shoots had a very similar succession of zones to the vigorous fruiting shoots previously observed in peach (Fournier *et al.*, 1998), a closely related species, while the structure of epicormic shoots has not been reported previously.

The axillary bud fate of a node has been linked to development rates (i.e. growth rate and plastochron) and also to the time of the season when a new node develops in proleptic shoots in peach (Génard *et al.*, 1994; Kervella *et al.*, 1995). In this study, sylleptic shoots were associated with high rates of shoot elongation. However, growth rate alone was not sufficient to explain the presence of sylleptic shoots because epicormic shoots had higher

growth rates than proleptic shoots, but epicormic shoots had lower probabilities of occurrence of sylleptic shoots than proleptic shoots. Therefore, factors other than growth rate must determine the differences in branching between proleptic and epicormic shoots. Seleznyova *et al.* (2012) also observed that the greater vigour in proleptic shoots developed after pruning of pear trees was not always correlated with more sylleptic shoots, and varied among cultivars. In addition, other factors, such as strong apical dominance, reduce branching. This phenomenon is determined by complex interactions of the hormones that control the outgrowth of axillary buds (Wilson, 2000; Cline and Dong-Il, 2002); however, this process is not yet fully understood, especially in perennials. In proleptic shoots, the zones with vegetative buds were associated with intermediate shoot growth rates, i.e. the rates were lower than those of the sylleptic shoot zones and higher than those of the zones of central flower buds and blind nodes. In both shoot types, the zones associated with the lowest growth rates developed blind nodes at the base of the shoots and blind nodes and nodes with central flower buds at the distal end of the shoots. The observation of axillary meristem differentiation into a reproductive organ (flower or inflorescence depending on the species) in the terminal third of the parent shoot has also been observed in apple and has been interpreted in this species as possibly resulting from a decrease in parent shoot elongation rate (Crabbé, 1987). The observation made in this study suggests that the variation in growth rate could matter for axillary meristem fate, but this association requires further investigation.

Even though the two water deficit treatments reduced shoot growth rates, the patterns of growth rates were similar among the treatments (Figs 4 and 6). In similar ways, the composition of proleptic and epicormic shoots (Figs 2A and 5A) and of each of their zones (Supplementary Data Table S1), in terms of relative frequencies of axillary meristem fates, were not significantly affected by the plant water status treatments. However, the numbers of nodes per proleptic shoot and per zone were reduced by water deficits. Thus, shoots with similar growth rate patterns developed shoot structures with similar proportions of axillary meristem fates and reductions in growth rate only reduced the number of nodes of certain zones and shoot types, especially those zones that had vegetative buds and sylleptic shoots. The effects of water deficit on the proportion of flower buds were not significantly different (Figs 2B and 5B), but differences were found within Zone 2 in both shoot types (Supplementary Data Table S2). These data only partially support previous research indicating that floral initiation on spurs occurs >3 weeks after the onset of hull split in 'Nonpareil' (Lamp *et al.*, 2001), and if water stress is applied during this period flower bud density is reduced (Goldhamer and Viveros, 2000). In the zone that mainly had flower buds that developed from axillary meristems (central flower buds), the proportion of nodes developing these flower buds was not reduced by water deficit in either shoot type (Supplementary Data Table S1). The formation of this bud type may be more related to its distal position on the shoot (Fournier *et al.*, 1998) and to the lower prevailing growth rate when this zone is developed (Kervella *et al.*, 1995) than to the two water deficit treatments.

The two water deficit treatments impacted the zone complexity of proleptic shoots by reducing the occurrence of Zone 1 and zone length by the development of fewer nodes in Zones 1 and 2. In contrast, vigorous epicormic shoots (Yamashita, 1971) had rapid growth rates, and therefore one might expect them to be more affected by water deficit than proleptic shoots, but only the occurrence of Zone 2 was affected by water deficit, and not the number of nodes per zone. The lesser effect of water deficit on node development on epicormic shoots compared with proleptic shoots may be related to shoot ontogeny. Epicormic shoots are thought to be the result of a reiteration process that repeats the basic structure of the part of the tree that was removed by pruning (Barthélémy and Caraglio, 2007). Thus, this type of shoot is ontogenetically younger than proleptic shoots. That is, the epicormic shoots grew from latent buds located directly on the structural branches of the trees that developed during the first season after planting in 2007; proleptic shoots instead grew from terminal buds of annual shoots formed in 2010 after a series of shoots had developed from planting in 2007. In addition, epicormic shoots that grew in response to the pruning of a branch may have had more resources available for shoot extension due to an imbalance between root and shoots caused by pruning (Mika, 1986), even under water stress conditions. Also, the development of epicormic shoots may be less influenced by lower plant water status than proleptic shoots, since the former shoot types tend to have lower xylem hydraulic resistance than the latter (Améglio *et al.*, 1997).

The overall mean number of nodes per shoot was affected by water deficit in proleptic shoots, since water deficits reduced the node numbers of Zones 1 and 2 and decreased the incidence of Zone 1. In epicormic shoots, by contrast, the number of nodes per shoot was not statistically reduced by water deficit even though Zone 2 was more frequently skipped. The reduction in nodes and zones of these shoots associated with water deficit may indicate a more rapid progression in tree ontogeny related to water deficits. If this is true, it is likely that the shoot development in subsequent years will be reduced in the water deficit trees even if they have similar plant water status to the trees in the high water status treatment.

In this study the central zones of proleptic and epicormic shoots were not always present even in the trees without water deficit (HWST). A lack of branching zones along with the reduction in the total number of nodes on shoots has been reported in peach (Fournier *et al.*, 1998) and apple (Renton *et al.*, 2006). However, in the present study shoots that lacked these zones in the HWST did not have significantly fewer nodes than shoots that had these zones (data not shown). Renton *et al.* (2006) found that the absence of the branching zones in apple corresponded with tree ontogeny, and, as trees got older, zones with axillary branching tended to disappear. The ontogenetic ageing of the proleptic shoots may explain why, in some cases, the zone with the highest probability of sylleptic shoots (Zone 2) was not present even in the HWST. The higher occurrence of the branching zone of epicormic shoots in comparison with proleptic shoots in the non-water-deficit treatment may be also explained by their different ontogenetic status due to the reiteration process of epicormic shoots. In addition, the lower occurrence of the branching zone in proleptic shoots in the HWST may also be a function of competition for resources between proleptic shoots on a branch (Mika, 1986).

In conclusion, this study documented three main points: (1) the structures of proleptic and epicormic shoots differed from each other in relation to their ontogenetic status within the trees; (2) the shoot structures were related to their growth rate patterns

during the growing season; and (3) water deficit impacted the structure of proleptic shoots more than that of epicormic shoots, but did not alter growth rate patterns during the season or their axillary meristem fate composition. These findings provide new understanding of the differences in shoot structures in almond trees and their responses to plant water status. These responses were similar to what has been observed previously due to tree age in apple trees. This suggests that water deficit may promote a more rapid progression in shoot ontogenetic stages and in tree physiological ageing. In addition, the lower response of epicormic shoots to water deficit potentially reflected the different ontogenetic characteristics of epicormic shoots compared with proleptic shoots, related to their reiteration status, and/or of having more resources available during their development.

SUPPLEMENTARY DATA

Supplementary Data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: axillary meristem fate for each zone of proleptic and epicormic shoots for each plant water status treatment. Table S2: number of flower buds per node for each zone of proleptic and epicormic shoots for each plant water status treatment.

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